(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number : **0 606 044 A1**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number : **93810835.4**

(22) Date of filing : **29.11.93**

(51) Int. Cl.[5] : **C07D 313/00**, A61K 31/365, C12P 17/08

(30) Priority : **04.12.92 GB 9225396**

(43) Date of publication of application : **13.07.94 Bulletin 94/28**

(84) Designated Contracting States : **AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE**

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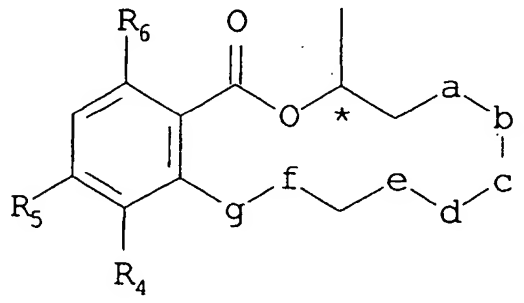
(54) **Lactones compounds useful as pharmaceuticals.**

(57) Novel compounds of formula I



I

wherein $R_4$, $R_5$, $R_6$, -a-b-, c, -d-e- and -f-g- are as defined are cytokine release inhibitors and IL-1 antagonists and are thus indicated for treatment of disorders with an aetiology associated with or comprising excessive cytokine release, particularly IL-1β release, including a wide variety of inflammatory states and diseases such as RA, osteoarthritis, septic shock, psoriasis, atherosclerosis, inflammatory bowel disease, Crohn's disease and asthma. Related known compounds Zearalenone and radicicol and derivatives thereof have also been found to have cytokine release inhibitor properties and have similar pharmaceutical applications.

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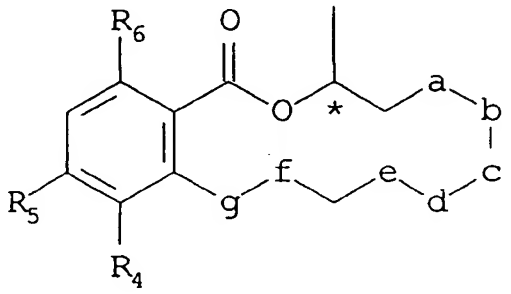
This invention relates to compounds useful as a cytokine release inhibitors and IL-1 antagonists. The invention provides the novel compounds of formula I



I

in which $R_4$ and $R_6$ are the same or different and are H, OH, $C_{1-4}$ alkoxy or $C_{1-4}$alkyl COO-,

$R_5$ is OH, $C_{1-4}$alkoxy or $C_{1-4}$alkyl COO,

one of -a-b- or -d-e- is $-CHR_7-CHR_8-$ and the other is cis- or trans- $-CR_7=CR_8-$, wherein $R_7$ and $R_8$ are the same or different and are H, OH, $C_{1-4}$ alkoxy or $C_{1-4}$alkyl COO-,

c is CH-OH or C=O and

-f-g- is $-CH_2-CH_2-$ or cis- or trans- -CH=CH-

provided that, when $R_4$ is H, $R_6$ is OH and -f-g- is trans- -CH=CH-; 1. $R_5$ is not OH when -a-b- is $-CH_2-CH_2-$, c is C=O and -d-e- is $-CH_2-CH_2-$, or 2. $R_5$ is not methoxy when -a-b- is $-CH_2-CH_2-$ or cis- -CH=CH- and c is C=O or CH-OH and -d-e- is -CHOH-CHOH,

in free form or base salt form or in the form of a physiologically-hydrolysable and -acceptable ester and wherein the asymmetric carbon marked * and the atoms a and/or b or d and/or e, when these are asymmetric carbon atoms have the R- or S-configuration or the compound comprises any mixture of the optical isomers thereof.

Preferably $R_4$ and $R_6$ are the same or different and are H, -OH, MeO- or Me-COO-. Preferably R5 is -OH, MeO- or MeCOO-. More preferably $R_4$ is H or MeO; $R_5$ is MeO, and $R_6$ is OH or MeO.

Preferably -a-b- is cis- or trans- $-CR_7'=CR_8'-$, wherein $R_7'$ and $R_8'$ are the same or different and are H, OH, MeO- or Me-COO-. More preferably -a-b- is cis- or especially trans- -CH=CH-.

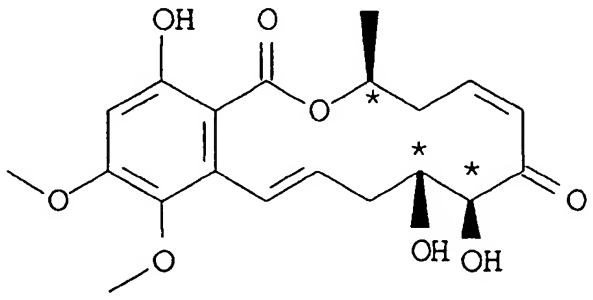
Preferably -d-e- is $-CHR_7'-CHR_8'-$, wherein $R_7'$ and $R_8'$ are as defined above. More preferably -d-e- is $-CH_2-CH_2-$ or especially -CHOH-CHOH-, wherein the OH groups may be in free or protected form.

Most preferably -f-g- is trans- -CH=CH-.

Preferably the asymmetric carbon atoms of the compounds of the invention all have the S-configuration.

In particular embodiments the invention provides compounds of formula I in which $R_4$ is H or methoxy, $R_5$ is methoxy, $R_6$ is OH, -a-b- is cis- or trans- -CH=CH-, c is CHOH or C=O, -d-e- is -CHOH-CHOH- and -f-g- is trans- -CH=CH-; provided that when -a-b- is cis -CH=CH-, then $R_4$ is methoxy and c is C=O in free form or base salt form or in the form of a physiologically-hydrolysable and -acceptable ester.

Formula I', which is formula I in which $R_4$ and $R_5$ are methoxy, $R_6$ is OH, -a-b- is cis- -CH=CH-, c is C=O, -d-e- is -CHOH-CHOH-, -f-g- is trans- -CH=CH- and the asymmetric carbon atoms marked * all have the S-configuration

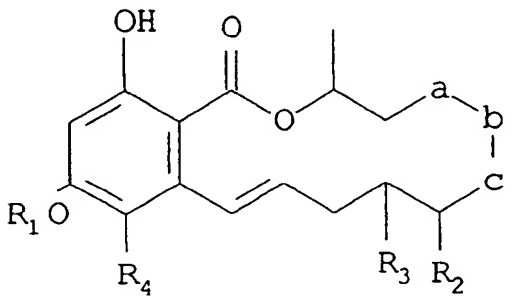


I'

is the structure which has been assigned to the novel metabolite designated 87-250904-F1 and having the following characteristics:

White needles (from methanol/water 1:1), m.p. 173-174°C,
$[\alpha]_D^{25}$ = -43.6°(MeOH, c = 0.76)
Mass spectrum (FAB) : m/e = 393 ($MH^+$)
IR spectrum in KBr : see Fig. 1
Proton NMR in $CDCl_3$, 360 MHz with TMS as internal standard, see Fig. 2
Solubility: Almost insoluble in water, readily soluble in methanol, DMSO, chloroform.
HPLC : Column : LiChrospher 100 RP-8 (5µm) (LiChroCART 125-4, Merck)
Mobile phase: acetonitrile/water/orthophosphoric acid 350:650:0.175 (by vol.)
Flow rate: 1.0 ml/min
Detection: UV 210 nm
Retention time: 3.8 min.

The compounds of formula I are novel compounds which belong to the same class of compounds as zearalenone, which is the compound of formula II

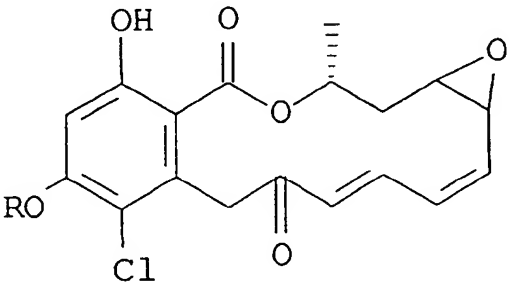



II

in which $R_1 = R_2 = R_3 = R_4 = H$, -a-b- = $-CH_2-CH_2-$, and c = C=O. Zearalenone is known to have anabolic effects. The compounds of formula II in which $R_1$ is methyl, $R_2 = R_3$ = OH, $R_4$ = H, -a-b- = $-CH_2-CH_2-$ or cis- -CH=CH- and c = C=O or CH-OH are described by Ellestad et al, J. Org. Chem. 43 2339 (1978), where they are stated to have "no particularly interesting activities" and it is speculated that the presence of the phenolic ether (i.e. the methoxy group on the benzene ring) may be responsible for this lack of activity.

In view of this it is surprizing that the compounds of formula I should have any pharmacological activity.

The compounds of formula I are also related to the compound radicicol, which is the compound of formula III




III

in which R is H. The corresponding methoxy compound in which R is methyl is also known.

Radicicol, a metabolite of Monosporium bonorden, is known to have antibiotic properties [Delmotte, Nature 171 344 (1953)]

Surprizingly, it has now been found that the compounds described above, namely the novel compounds of Formula I, radicicol, O-methyl radicicol and the zearalenone derivatives described by Ellestad et al also have cytokine release inhibitor properties, in particular, they inhibit the release of IL-1, IL-6 and TNF-α, and also act as functional antagonists of IL-1.

The compound of formula I' may be produced by cultivating a producing microbial strain in a nutrient medium. Preferred micro-organisms are strains of pycnidial imperfect fungi, in particular the strain F/87-250904, which produces the metabolite 87-250904-F1.

This strain has been isolated from an unidentified lichen collected in South Africa, and a viable culture of the strain was deposited on 6 Nov.1991 at the ARS Patent Culture Collection, US Dept. of Agriculture, Northern

Regional Research Center, Peoria, Illinois, USA under the provisions of the Budapest Treaty and was given the reference number NRRL 18919. The culture may also be obtained from Sandoz Ltd., Basle, Switzerland.

The fungal strain NRRL 18919 grows on most usual fungal agar media such as 2 % malt extract agar. The temperature range for growth is between approx. 5 and 37°C, the optimal temperature for growth is between approx. 24 and 32°C. On 2% malt extract agar in petri dishes and at 27°C strain NRRL 18919 will form after 10 days incubation colonies 25 to 35 mm in diameter. The colonies appear brown to greenish black to black with moderately developed aerial mycelium. No pronounced diffusable pigments are formed under these conditions. Strain NRRL 18919 can degrade starch and keratin, but not or only to a very limited degree cellulose and chitin.

The pycnidia of strain NRRL 18919 are very variable in shape and size. They range from approx. 30µ large, globose to pyriform with a single ostiolum to irregularly shaped, up to 600µ large pycnidia or pycnidial complexes with several ostioli. The outer pycnidial walls are composed of brown cells. The conidia are hyaline, aseptate, bacilliform, reniform to allantoid, often constricted and 3.0 - 5.5 x 1.0 - 1.7µ large. Conidia are produced within the pycnidia by slender phialides and collect outside the ostioli in a whitish mass. The strain NRRL 18919 can best be accomodated in the genus Phoma Sacc. and fits quite well to the description of Phoma cava Schulzer.

The new strain NRRL 18919 may be cultured at suitable temperatures in various culture media using appropriate nutrients and mineral substances, as aerobic surface or immersion cultures. The invention also provides fermentation broths which are obtained by cultivation of an 87-250904-F1 producing fungal strain, particularly of the strain NRRL 18919. A further aspect of the invention provides a process for the preparation of the compound of Formula I comprising the steps of cultivating an 87-250904-F1-producing fungal strain and isolating the metabolite 87-250904-F1 which is formed.

The fermentation media should contain a utilisable source of carbon and optionally mineral salts and growth factors, all of which can be added in the form of well defined products or as complex mixtures, as are found in biological products of various origins.

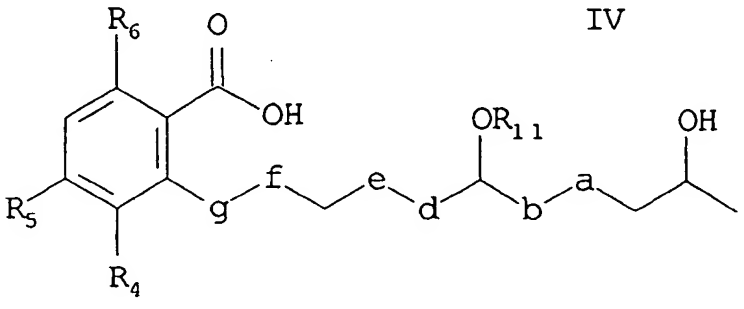
In order to produce the new metabolite 87-250904-F1, strains may also be used which are obtained by selection or mutation under the influence of ultra-violet radiation, X-rays or by other means, e.g. by the use of chemical mutagens.

As soon as a sufficient amount of metabolite has accumulated in the culture, it may be concentrated and isolated in conventional manner, for example by extraction and subsequent chromatographic methods.

Compounds of formula I in which a-b is trans- -CH=CH- may be prepared from the corresponding compounds in which a-b is cis- -CH=CH- by isomerization under mild alkaline conditions, for example in solution in pyridine, suitably for 12 - 48 hr at temperatures between 0 and 80°C, preferably at about 50°C.

The compounds of formula I may also be prepared by complete or partial chemical synthesis using conventional synthesis techniques.

Thus in a further aspect the invention provides a process for the preparation of a compound of formula I, in which c is CHOH, which comprises cyclising a compound of formula IV

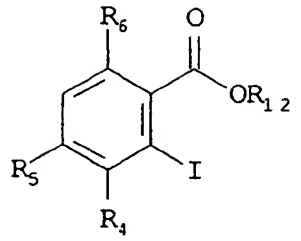
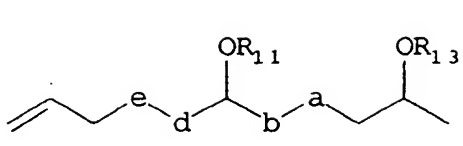



IV

wherein $R_4$, $R_5$, $R_6$, -a-b-, -d-e- and -f-g- are as defined above with the exception that any OH substituents on -a-b- or -d-e- are in suitably protected form, and $R_{11}$ is H or an OH protecting group;
and removing any OH protecting groups therefrom.

Compounds of formula I in which c is C=O may be prepared from the the cyclised product by oxidation of the CHOH group at c.

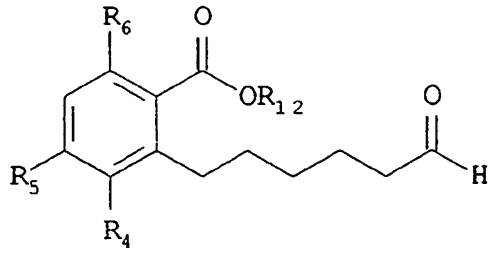
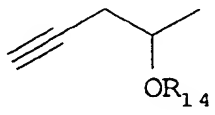
The compounds of formula IV wherein -f-g- is trans- -CH=CH- may be prepared by linking compounds of formulae V and VI

V  +  VI

wherein $R_4$, $R_5$, $R_6$, $R_{11}$, -a-b-, -d-e- and -f-g- are as defined above for formula IV and $R_{12}$ and $R_{13}$ are OH protecting groups,
and removing the $R_{12}$ and $R_{13}$ are OH protecting groups.

Compounds of formula VI may be prepared by linking an hydroxy protected analogue of 4-hydroxybut-1-yne with hex-1-en-5-one, partially or completely reducing the acetylene bond and, if appropriate, adding an $R_{11}$ OH protecting group.

The compounds of formula IV wherein -f-g- is $-CH_2-CH_2-$ may be prepared by linking compounds of formulae VII and VIII

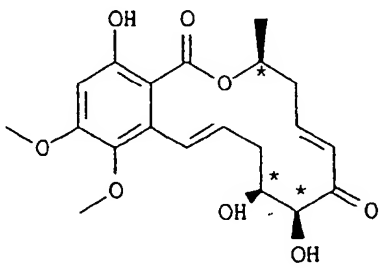
VII  +  VIII

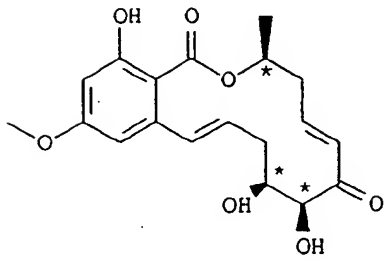
wherein $R_4$, $R_5$, $R_6$, and $R_{12}$ are as defined above for formulae IV and V and $R_{14}$ is an OH protecting group,
partially or completely reducing the acetylene bond and if appropriate removing OH protecting groups.

The compound of formula VII may be prepared by linking a compound of formula V as above with an hydroxy protected analogue of 6-hydroxy-hex-1-ene, reducing the alkylene bond corresponding to f-g in formula I, removing the protecting group from the terminal hydroxy group on the $C_6$ side chain and oxidising this hydroxy group to an aldehyde group.

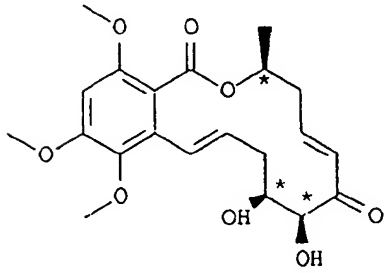
The processes described above may be carried out using conventional synthesis procedures, reagents and conditions; for instance, as described hereinafter in the Examples.

Preferred compounds in which a-b is trans- -CH=CH- are the compounds of formulae I", X and XI.

 I"

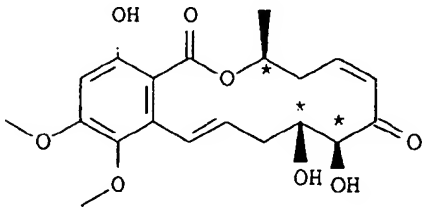


X

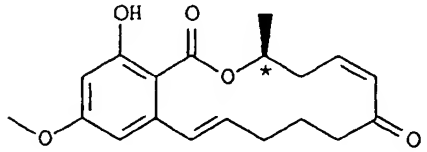


XI

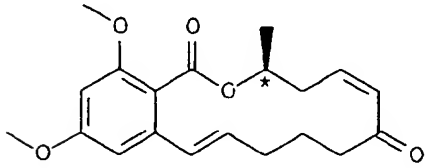
Preferred compounds in which a-b is cis- -CH=CH- are the compounds of formulae I', XII and XIII.



I'



XII



XIII

The compounds of the invention have pharmacological properties. In particular, they show cytokine inhibitory effects, acting not only to inhibit the release of IL-1, IL-6 and TNF-α, but also as functional antagonists of IL-1 as indicated in the following in vitro and in vivo test methods:

1. Cytokine Release from THP-1 Cells

The THP-1 cell line is generally available and is described by Tsuchiya et al, Int. J. Cancer 26 171-176 (1980). 900 µl THP-1 cells (0.5 x $10^6$ cells) together with 100 U γ-interferon/0.9 ml RPMI 1640 medium (containing 2 mM L-glutamine and 5 % heat-inactivated foetal calf serum) are pipetted into 24 well culture plates. 100 µl of the compound to be tested are then added. After 3 hours at 37°C in 5 %

$CO_2$/95 % air, 10 µl lipopolysaccharide 500 µg/ml is added and the incubation continued for a further 40 hours. Appropriate controls (with and without stimulus, solvent) are also included. The media are then removed and clarified by centrifugation at 1000 g for 10 min. 1.0 ml digitonin 0.01 % is added to the wells to lyse the cells which are loosened by scraping with a rubber policeman and left at 4°C for 10 min. Lactate dehydrogenase measurements are then performed immediately and the samples stored at -20°C until the other determinations can be made. The assays are: IL-1β (medium and lysate), IL-6 (medium), TNF-α (medium), PGE2 (medium and lysate), lactate dehydrogenase (LDH) (medium and lysate) and DNA (lysates) . IL-1β, IL-6 and TNF-α assays are determined using commercially available ELISA kits (Cistron), PGE2 is measured using a standard RIA and DNA fluorimetrically using DAPI (4',6-diamidino-2-phenylindole.2HCl)

In this test, the compounds of the invention inhibit IL-1β ,Il-6, TNF-α and PGE2 release at a concentration of about 0.001 to 10 µM. In contrast DNA levels remain substantially unaffected, and the compounds are non-toxic, since LDH release is unchanged.

2. Cytokine Release from Human Monocytes

a) Human Monocytes

Mononuclear cells are obtained from the blood of healthy volunteers via centrifugation and cultivated on tissue culture dishes with the test compound at various concentrations [Schnyder et al., Agents & Actions, **30**, 350-362 (1990)]. The non-adherent lymphocytes are removed after 4 hr. by washing several times. Fresh medium, test compound and LPS (10 µg/ml) as stimulant are added and the monocytes incubated for a further day. The pooled culture media are diluted 1:10 with fresh medium and added to confluent rabbit chondrocytes. Metallo- proteinase (MP) activity in the chondrocyte culture medium is assayed after a further 2 days as described below. Compounds of the invention are active in suppressing monokine release in this test method at a concentration of the order of from 0.001 to 10µM.

b) Determination of IL-1 by the chondrocyte test

Purified IL-1, recombinant human IL-1-β (rhIL-1) or conditioned media collected from stimulated human monocytes, mouse macrophages or mouse cell line $P388D_1$, cause characteristic changes in the secretory pattern of chondrocytes. In particular, a latent metalloproteinase (MP) is induced, whilst secretion of plasminogen activator is reduced. The property of the metallo-proteinase or stromelysin has been described in detail [Chin et al., J. Biol. Chem. **260**, 12367 - 12376 (1985)], as has that of the plasminogen activator [Schnyder et al., Analyt. Biochem. **200**, 156-162, 1992]. Dose-response curves using purified or recombinant IL-1 and neutralisation with an antibody to human monocyte IL-1 have shown that this system can be used as a specific and sensitive bioassay for IL-1. Stimulation of the secretion of a latent metalloproteinase by rabbit articular chondrocytes is relatively IL-1-specific, IL-2, TNF-α, recombinant human interferon-α, and -γ, phorbol myristate acetate, Concanavalin A, E-type prostaglandin and indomethacin having no influence [Schnyder and Payne, Brit. J. Rheumatol. **24** (suppl. 1), 128 - 132 (1985); Schnyder et al., J. Immunol. **138**, 496 - 503 (1987)].

Chondrocytes are harvested and cultured as described [Evequoz et al., Biochem. J. **219**, 667 - 677 (1984)]. Briefly, chondrocytes are released from slices of distal femur articular cartilage from ca. 1.2 kg female New Zealand White rabbits by treatment with proteinases. The washed cells are cultured on 48-well culture plates in DMEM, enriched with 1 % antibiotics, 2 mM glutamine and 10 % heat-inactivated fetal calf serum. After reaching confluency the cells are incubated with 20 µl samples of the test culture media for IL-1 bioassay and made up to a volume of 300 µl Iscove's modified Dulbecco's medium. The supernatant media are collected after 48 h, centrifuged and processed for biochemical analysis.

c) Biochemical Assays

Metalloproteinase (MP) is measured kinetically by using a 96-well plate Twinreader (Flow Laboratories AG) linked to a personal computer. Ac-Pro-Leu-Gly-S-Leu-Leu-Gly-OC2H5, a synthetic substrate for vertebrate collagenase is used for the determination of MP [Weingarten and Feder, Anal. Biochem. 147, 437-440 (1985)]. 50 µl of the latent MP is activated with 50 µl trypsin (120 µg/ml in 50 mM PIPES pH 6.8, containing 20 mM CaCl2) for 30 min at 37°C, after which time the activities of all serine proteinases are stopped by adding 150 µl Soybean trypsin inhibitor (SBTI; 166 µg/ml in the above buffer). A 50 µl aliquot of the activated MP is then mixed with 100 µl reagent solution (2.5 mM 5,5'-dithio-bis-2-nitrobenzoic acid; DTNB, 100 µg/ml SBTI, 20 mM CaCl2 in the above buffer), and kept for 10 min at room temperature in order to react with all free SH-groups. The reaction is then started by adding 100 µl substrate solution (1.25 mM in buffer containing 100 µg/ml SBTI) and the changes in absorbance at 414 nm is measured 11 times at 1 min intervals.

Example 8: The compound of formula I in which $R_4$ is H, $R_5$ and $R_6$ are OMe, -a-b- is cis -CH=CH-, c is C=O and -d-e- and -f-g- are $-CH_2-CH_2-$

a). 4-dimethyl,t-butyl-silyloxy-pent-1-yne

17.8 g of (±)-4-pentyn-2-ol is dissolved in 150 ml of acetonitrile with stirring at room temperature, 15.8 g of imidazole followed by 35 g of dimethyl,t butyl-silyl chloride are added giving rise to an exothermic reaction whereby all components of the reaction mixture initially go into solution but afterwards a precipitate immediately forms. The mixture is stirred overnight at room temperature after which it is suction filtered, the liquid fraction concentrated and a further 2.9 g of imidazole and 6.3 g of dimethyl,t butyl-silyl chloride are added. After 3 hr the reaction mixture is again suction filtered, the liquid fraction concentrated subjected to fractional distillation. The title product is in the first three fractions which are combined and the product purified by distillation at reduced pressure (12 mm Hg) at 64-68°C.

b). The product of formula VII in which $R_4$ is H, $R_5$ and $R_6$ are OMe and $R_{12}$ is Me

14.6 g of the corresponding compound of formula V, 14.6 g of the product of step a) of this Example, 0.3 g of palladium diacetate, 1.1 g of tri o-tolyl phosphine and 7.6 g of silver acetate are added to 60 ml of tetrahydrofuran and the mixture warmed with stirring to 70°C in an oil bath. After 48 hr, at which time the reaction is about 70% completed as judged by thin layer chromatography, a further 0.3 g of palladium diacetate and 1.1 g of tri o-tolyl phosphine are added and the mixture warmed over the weekend at 70°C. The mixture is then suction filtered over Hyflo, concentrated under high vacuum and purified by silica gel chromatography (hexane/ethyl acetate 4:1) to give a first intermediate product, i.e. an analogue of the corresponding compound of formula VII in which $R_6$ = OMe, $R_5$ = OMe, $R_4$ = H, $R_{12}$ = Me and the aldehyde group of the $C_6$ side chain is a dimethyl,t-butyl-silyl-protected OH group.

6.7 g of this first intermediate product is dissolved in 100 ml of methanol, 500 mg of palladium/charcoal catalyst containing 10% palladium (Pd/C 10%) is added and the mixture hydrogenated under stirring for 48 hr (350 ml of $H_2$ being taken up after the first 24 hr). The reaction mixture is then suction filtered over Hyflo and concentrated; though at this stage it is found that the $C_6$ side chain double bond is only 30% reduced as judged by NMR. The reaction product is, therefore again taken up in 150 ml of methanol, 500 mg of the Pd/C 10% catalyst is added and the mixture hydrogenated over the weekend at room temperature. The resultant reaction mixture is suction filtered over Hyflo, concentrated and a second intermediate product, i.e. an analogue of the corresponding compound of formula VII in which the aldehyde group of the $C_6$ side chain is an OH group, is separated from the corresponding siloxy-protected compound by silica gel chromatography (hexane/ethyl acetate 2:1) in a yield of 4.9 g.

1.2 ml of oxalyl chloride is added to 30 ml of dichloromethane stirred at room temperature, the mixture cooled to -60°C and 1.9 ml of DMSO in 7 ml of dichloromethane added dropwise over a 5 min period after which the mixture is stirred for a further 3 min and a solution of 3.5 g of the second intermediate product of this example in 20 ml of dichloromethane is added dropwise over a 10 min period. A precipitate forms, the mixture is stirred for a further 20 min, concentrated and 8.2 ml of triethylamine is added over a 5 min period. A thick paste precipitate forms and is allowed to accumulate at room temperature for 1.5 hr. The solid product is extracted with dichloromethane, washed twice with 10% citric acid solution, once each with saturated brine and $NaHCO_3$, dried over $Na_2SO_4$ and concentrated to give 3.6 g of the title product in the form of gum.

c). The product of formula IV in which in which $R_4$ is H, $R_5$ and $R_6$ are OMe, -a-b- is cis -CH=CH-, -d-e- and -f-g- are $-OH_2-OH_2-$ and $R_{11}$ is H

0.39 g of the product of step a) of this Example is added to 5 ml of tetrahydrofuran under an atmosphere of nitrogen, 1.2 ml of butyl lithium is added dropwise over a 2 min period at -70°C. After 1 hr a solution of 0.5 g of the final product of step b) of this Example in 4 ml of tetrahydrofuran is added dropwise over a 5 min period, the mixture stirred at -70°C for 1.5 hr and then allowed to warm to room temperature. At this point the reaction is complete as judged by thin layer chromatography. Whilst cooling with ice, the product is washed with 10% citric acid solution, extracted three times with ethyl acetate, washed with saturated brine and $NaHCO_3$, dried over $Na_2SO_4$ and concentrated to give 0.87 g of a resinous first intermediate product, i.e. an analogue of the corresponding compound of formula IV in which the COOH substituent is in the form of the methoxy ester, the bond corresponding to the -a-b- bond of the compound of formula I is an acetylene bond and the OH substituent on the $C_{10}$ carbon atom of the $C_{11}$ side chain is t-butyl-silyl-protected.

3.1 g of this first intermediate product is dissolved in 50 ml of pyridine, 200 mg of 10% Pd/$BaSO_4$ is added and the mixture hydrogenated at room temperature with magnetic stirring. After 4 hr greater than the theoretical hydrogen requirement (240 ml) has been taken up. The resultant product is then sampled, concentrated, taken up in dichloromethane and washed with 10% citric acid solution (at this point the re-

action is judged to be complete by NMR). The product is separated from the catalyst by suction filtration over Hyflo, concentrated, taken up in ethyl acetate, washed with saturated brine, dried over $Na_2SO_4$ and concentrated to give 3.05 g of a resinous second intermediate product, i.e. the analogue of the first intermediate product of this step in which the bond corresponding to the -a-b- bond of the compound of formula I is cis -CH=CH-.

By analogy with step f) of Example 6 the second intermediate product of this step is converted into the title product (m/z: 367(MH+), 349(40), 331(100), 305(90), 191(100)).

d). The compound of formula I in which $R_4$ is H, $R_5$ and $R_6$ are OMe, -a-b- is cis -CH=CH- c is C=O and -d-e- and -f-g- are $-CH_2-CH_2-$

1.7 g of the final product of step c) above is dissolved with stirring in 1.8 1 of acetonitrile, 5.9 g of 2-chloropyridine iodide and 6.5 ml of triethylamine are added and the mixture is warmed over the weekend to 50°C in an oil bath with stirring. The product is then concentrated, taken up in ethyl acetate, washed with 10% citric acid solution $NaHCO_3$ and saturated brine, dried over $Na_2SO_4$ and concentrated to give 1.6 g of a crude intermediate product (i.e. the analogue of the title product in which c is CHOH) in the form of a brown resin. This crude product is purified by silica gel chromatography (hexane/ethyl acetate 3:2), using dichloromethane to dissolve the intermediate product.

To 2 ml of dichloromethane at room temperature is added 5 µl of oxalyl chloride, the mixture cooled to -70°C and a solution of 93 µlof DMSO in 1 ml of dichloromethane is added dropwise over 3 min with stirring. The mixture is stirred for a further 5 min after which a solution of 0.2 g of the intermediate product of this step is added dropwise over 5 min. A precipitate forms, the mixture is stirred for a further 15 min, 0.4 ml of triethylamine is added dropwise over 5 min, the mixture stirred for 1 hr at -70°C and then allowed to warm to room temperature over 1 hr. The resultant product mixture is diluted with dichloromethane, washed twice with 10% citric acid solution, once with saturated brine, dried over $Na_2SO_4$, concentrated and purified by silica gel chromatography and recrystallisation to give about 100 mg of pure product (m. pt. 110°C).

Example 9: The compound of formula I in which $R_4$ and $R_6$ are H, $R_5$ is OMe, -a-b- is trans -CH=CH- c is C=O and -d-e- and -f-g- are $-CH_2-CH_2-$

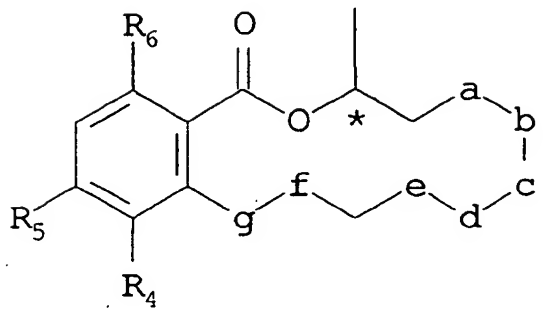
35 mg of magnesium is stirred with 1 ml of diethylether and 1 ml of benzene and 0.18 g of iodine is added, the dark brown colour dissipating after 1.5 hr. The liquid phase of the resultant mixture is added to a solution of 50 mg of the final product of Example 6 in 2 ml of benzene with the resultant formation of a precipitate. The mixture is stirred at 60°C for 2.5 hr, the product taken up in ethyl acetate, washed with 1N HCl saturated $NaHCO_3$ and saturated brine, dried over $Na_2SO_4$, concentrated and purified by silica gel chromatography (toluene/methanol 98:2). The product is further purified on a silica gel column (hexane/ethyl acetate 3:2) to give about 30 mg of the pure title product in the form of an oil (m/z: 333(MH+), 315(80), 265(100), 177(90).

Example 10: The compound of formula I in which $R_4$ is H, $R_5$ and $R_6$ are OMe, -a-b- is trans -CH=CH- c is C=O and -d-e- and -f-g- are $-CH_2-CH_2-$

By analogy with Examples 3 and 9 the title product is prepared form the corresponding product in which -a-b- is cis -CH=CH-, i.e. the final product of Example by isomerisation of the -a-b- bond. The title product is obtained in the form of an oil (m/z: 346(M+), 205(50), 191(100), 178(40), 152(40, 95(55).

## Claims

1. A compound of Formula I



I

in which $R_4$ and $R_6$ are the same or different and are H, OH, $C_{1-4}$ alkoxy or $C_{1-4}$alkyl COO-,

$R_5$ is OH, $C_{1-4}$alkxoy or $C_{1-4}$alkyl COO-,

one of -a-b- or -d-e- is $-CHR_7-CHR_8-$ and the other is cis- or trans- $-CR_7=CR_8-$, wherein $R_7$ and $R_8$ are the same or different and are H, OH, $C_{1-4}$ alkoxy or $C_{1-4}$alkyl COO-,

c is CH-OH or C=O and

-f-g- is $-CH_2-CH_2-$ or cis- or trans- -CH=CH-

provided that, when $R_4$ is H, $R_6$ is OH and -f-g- is trans- -CH=CH-; 1. $R_5$ is not OH when -a-b- is $-CH_2-CH_2-$, c is C=O and -d-e- is $-CH_2-CH_2-$, or 2. $R_5$ is not methoxy when -a-b- is $-CH_2-CH_2-$ or cis- -CH=CH- and c is C=O or CH-OH and -d-e- is -CHOH-CHOH-,

in free form or base salt form or in the form of a physiologically-hydrolysable and -acceptable ester and wherein the asymmetric carbon marked * and the atoms a and/or b or d and/or e, when these are asymmetric carbon atoms have the R- or S-configuration or the compound comprises any mixture of the optical isomers thereof.

2. A compound according to claim 1 in which
$R_4$ and $R_6$ are the same or different and are H, -OH, MeO- or Me-COO-, $R_5$ is -OH, MeO- or MeCOO-, -a-b- is cis- or trans- $-CR_7'=CR_8'-$, wherein $R_7'$ and $R_8'$ are the same or different and are H, OH, MeO- or Me-COO- and -d-e- is $-CHR_7'-CHR_8'-$, wherein $R_7'$ and $R_8'$ are as defined.

3. A compound according to claim 2, in which
$R_4$ is H or MeO, $R_5$ is MeO, $R_6$ is OH or MeO, -a-b- is cis- or trans- -CH=CH-, -d-e- is $-CH_2-CH_2-$ or -CHOH-CHOH- and -f-g- is trans- -CH=CH-.

4. A compound of formula 1 in which
$R_4$ is H or methoxy, $R_5$ is methoxy, $R_6$ is OH, -a-b- is cis- or trans- -CH=CH-, c is CHOH or C=O, -d-e- is -CHOH-CHOH- and -f-g- is trans- -CH=CH-; provided that when -a-b- is cis -OH=OH-, then $R_4$ is methoxy and c is C=O in free form or base salt form or in the form of a physiologically-hydrolysable and -acceptable ester.

5. A compound according to any of the preceding claims, in which a-b is trans- -CH=CH-.

6. A compound according to any of the preceding claims, in which the asymmetric carbon atoms all have the S-configuration.

7. A compound of formula I', I'', X, XI, XII, or XIII as hereinbefore defined.

8. A process for the preparation of the compound of formula I' comprising the steps of cultivating an 87-250904-F1-producing fungal strain and isolating the metabolite 87-250904-F1 which is formed.

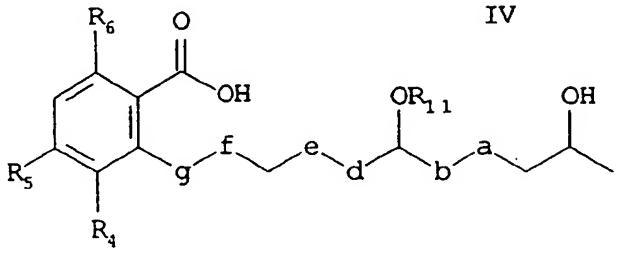
9. A process according to Claim 8 in which the fungal strain is NRRL 18919.

10. A pure culture of fungal strain NRRL 18919.

11. A fermentation broth which is obtained by cultivation of the fungal strain NRRL 18919.

12. A process for the preparation of a compound of formula I in which a-b is trans- -CH=CH- comprising the step of isomerization of the corresponding cis- isomer under mild alkaline conditions.

13. A process for the preparation of a compound of formula I, in which c is CHOH, which comprises cyclising a compound of formula IV



IV

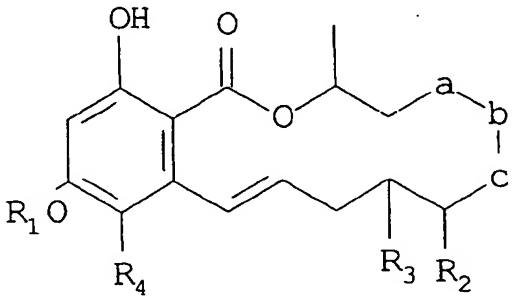
wherein $R_4$, $R_5$, $R_6$, -a-b-, -d-e- and -f-g- are as defined in claim 1 with the exception that any OH substituents on -a-b- or -d-e- are in suitably protected form, and $R_{11}$ is H or an OH protecting group; and removing any OH protecting groups therefrom.

14. A process for the preparation of a compound of formula I, in which c is C=O comprising oxidising the c CHOH group of a compound of formula I wherein c is CHOH.

15. A method for the treatment of disorders with an aetiology associated with or comprising excessive cytokine release, particularly IL-1β release, such as rheumatoid arthritis, osteoarthritis, septic shock, psoriasis, atherosclerosis, inflammatory bowel disease, Crohn's disease and asthma, in a subject in need thereof, which method comprises administering to said subject an effective amount of

a) a compound of Claim 1; or

b) a compound of formula II



II

in which:

$R_1$ is H or methyl;

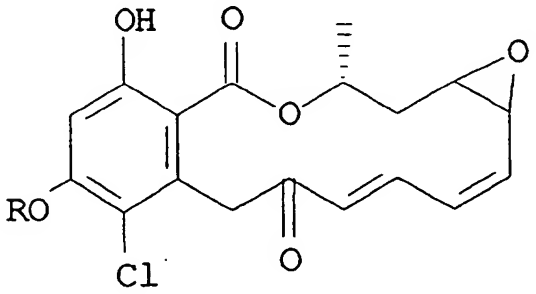
$R_2$ and $R_3$ are H or OH,

$R_4$ is H or methoxy;

-a-b- is $-CH_2-CH_2-$ or -CH=CH-

and c is C=O or CH-OH (other than a compound of Claim 1); or

c) a compound of formula III



III

in which R is H or methyl
in free or base salt form, or a physiologically-hydrolysable and -acceptable ester thereof.

16. A compound of formula II, stated in Claim 15, in which:
$R_1$ is methyl;
$R_2$ and $R_3$ are H or OH,
$R_4$ is H or methoxy;
-a-b- is $-CH_2-CH_2-$ or cis- -CH=CH-;
and c is C=O or CH-OH;
in free base or salt form, or a physiologically-hydrolysable and -acceptable ester thereof for use as a pharmaceutical.

17. A pharmaceutical composition comprising a compound according to claim 1.

18. A pharmaceutical composition comprising a compound of formula II, stated in Claim 15, in which:
$R_1$ is methyl; $R_2$ and $R_3$ are H or OH; $R_4$ is H or methoxy;-a-b- is $-CH_2-CH_2-$ or cis- -CH=CH-; and c is C=O or CH-OH; in free base or salt form, or a physiologically-hydrolysable and -acceptable ester thereof.

FIG. 1

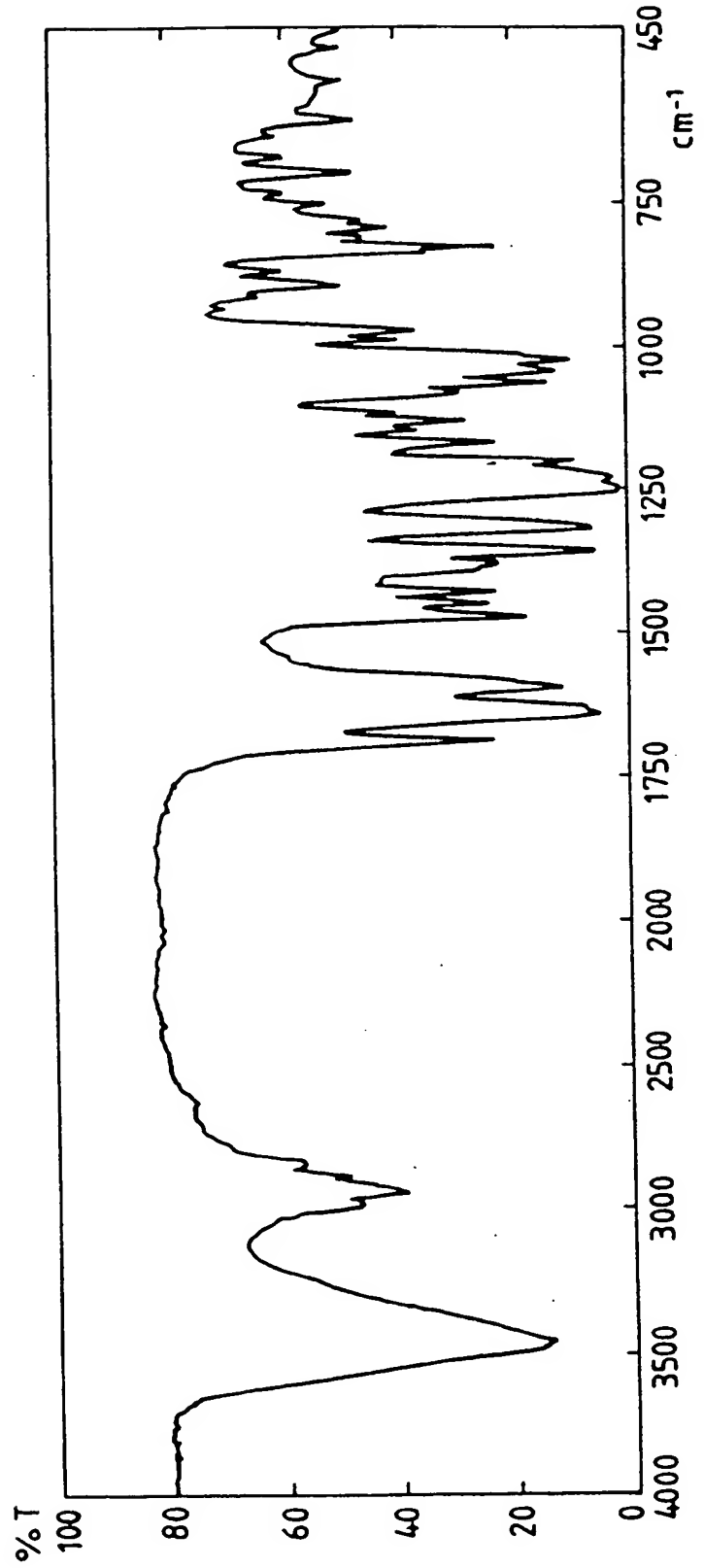
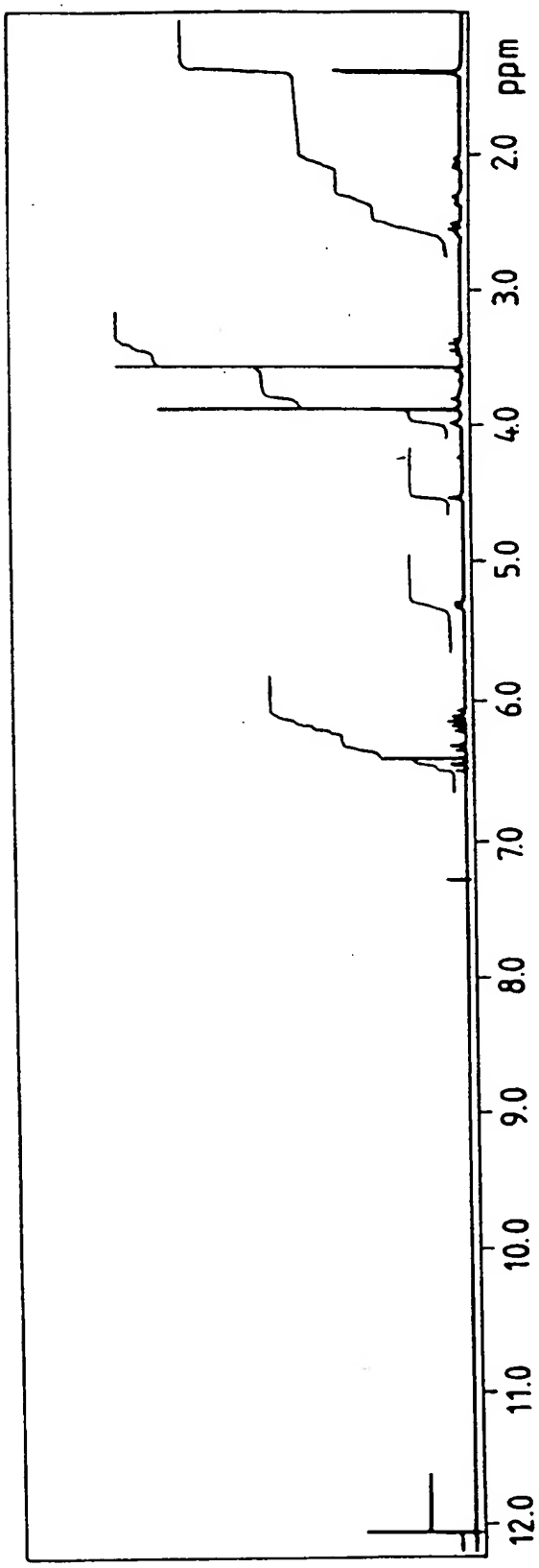

% T
100
80
60
40
20
0
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
450
cm-1

FIG. 2



European Patent Office

# PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 93 81 0835

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.5) |
|---|---|---|---|
| A | US-A-3 196 019 (F.N. ANDREWS ET AL.)<br>* column 3 - column 8 *<br>--- | 1 | C07D313/00<br>A61K31/365<br>C12P17/08 |
| A | HELVETICA CHIMICA ACTA<br>vol. 55, no. 8 , 1972 , BASEL CH<br>pages 3030 - 3048<br>G. BOLLIGER, CH. TAMM '306. VIER NEUE METABOLITE VON GIBERELLA ZEAE.'<br>* page 3030 - page 3032; example 8 *<br>--- | 1,4,5,7 | |
| A | JOURNAL OF ORGANIC CHEMISTRY.<br>vol. 43, no. 12 , 1978 , EASTON US<br>pages 2339 - 2343<br>G. ELLESTAD ET AL. 'NEW ZEARALENONE RELATED MACROLIDES AND ISOCOUMARINS FROM AN UNIDENTIFIED FUNGUS.'<br>* page 2339 - page 2343 *<br>----- | 1,4,7-11 | |

TECHNICAL FIELDS SEARCHED (Int.Cl.5)

C07D
C12P

## INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 23 March 1994 | Francois, J |

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
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L : document cited for other reasons

& : member of the same patent family, corresponding document

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-C-

Remark: Although claim 15 is directed to a method of treatment of the human/animal body (Art. 52(4) EPC) the search has been carried out and based on the alleged effects of the compound/composition